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Correlation of B7-H3 with androgen receptor, immune pathways and poor outcome in prostate cancer: an expression-based analysis

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Abstract

BACKGROUND: *B7-H3 (CD276)*, part of the B7 superfamily of immune checkpoint molecules, has been shown to have an immunomodulatory role. Its regulation, receptor and mechanism of action remain unclear. B7-H3 protein expression correlates with prostate cancer outcomes, and humanized monoclonal antibodies (that is, enoblituzumab) are currently being investigated for therapeutic use. Here we used genomic expression data to examine the relationship between *B7-H3* mRNA expression and prostate cancer.

METHODS: Prostatectomy tissue from 2781 patients were profiled using the Affymetrix HuEx 1.0 ST microarray. Pairwise comparisons were used to identify significant associations between B7-H3 expression and clinicopathologic variables, and survival analyses were used to evaluate the prognostic significance of *B7-H3*. Pearson's correlation analyses were performed to assess the relationship of *B7-H3* expression with molecular subtypes and individual transcripts. Androgen

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

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receptor (AR) occupancy at the *B7-H3* locus was determined using chromatin immunoprecipitation (ChIP), and androgen-dependent expression changes in *B7-H3* was evaluated by quantitative reverse transcription PCR in LNCaP cell lines. Oncomine was queried to evaluate *B7-H3* expression in metastatic disease.

RESULTS: *B7-H3* mRNA expression was positively associated with higher Gleason score (P <0.001), tumor stage (P <0.001), and castrate resistant metastatic disease (P <0.0001). High *B7-H3* expression correlated with the development of metastasis and prostate cancer specific mortality, but this was not significant on multi-variable analysis. *B7-H3* expression correlated with ERG-positive disease (r = 0.99) and *AR* expression (r = 0.36). ChIP revealed an AR-binding site upstream of *B7-H3*, and the presence of androgens decreased *B7-H3* expression in LNCaP suggesting potential direct AR regulation. Gene set enrichment analysis demonstrated an association of *B7-H3* with androgen signaling as well as immune regulatory pathways.

CONCLUSIONS: Higher *B7-H3* expression correlates with Gleason grade, prostate cancer stage and poor oncologic outcomes in prostatectomy cohorts. *B7-H3* expression appears to be related to androgen signaling as well as the immune reactome.

INTRODUCTION

T-cell activation requires engagement of the T-cell receptor but additionally engagement of co-stimulatory molecules, most notably CD28 that binds B7–1 and B7–2 on antigen presenting cells. Several molecules sharing homology to B7 have been identified and constitute a B7 superfamily.[1] Among these molecules, B7-H1 (PD-L1) has been shown to have an important role as an immune checkpoint ligand within the tissue micro-environment and can be targeted by humanized antibodies to allow for anti-tumor responses in several malignancies including advanced melanoma, lung, bladder and renal cancers.[2] B7-H3 (CD276) was identified from a human dendritic-cell-derived cDNA library and shares roughly 20–27% amino-acid identity with other B7 family members.[3]

B7-H3 is expressed in multiple tissue types, including the epithelial cells of tumors. Expression is also inducible on the surface of T cells, dendritic cell and monocytes.[3] The receptor, regulation and mechanism of action of B7-H3 are not fully known, but recent preclinical studies suggest varied effects of B7-H3 depending on the mechanism of inflammation and involved T-cell subset.[4] In their work, utilizing a B7-H3 knockout model and various modes of inflammation, Luo *et al.*[4] determined that although B7-H3 may inhibit Th2 type responses, it may acts as a co-stimulatory molecule for Th1 and Th17 type responses. Th1 responses serve to regulate CD8+ T cells and are thought to be critical for recognition of tumor antigens. In line with the findings above, expression of B7-H3 in tumors promotes tumor regression and knockout of B7-H3 increases tumor growth in mouse models of spontaneous cancer.[5–7]

At the protein level, B7-H3 expression has been investigated in various malignancies, most notably prostate cancer. Utilizing immunohistochemistry, Roth *et al.*[8] demonstrated that B7-H3 is expressed in normal prostate epithelium and is more intensely expressed by prostate cancer. Within their series, virtually all prostate cancer expressed B7-H3, however, the intensity of expression varied with cancers exhibiting more aggressive phenotypes (larger

tumors, those with extraprostatic extension) expressing higher levels of the protein. In addition, elevated levels of B7-H3 expression correlated with disease progression following surgery. These results were supported by work from Zang *et al.*,[9] which also demonstrated increased expression of B7-H3 in the majority of prostate cancers, particularly in tumors that were non-organ confined at surgery. In their work, strongly positive B7-H3 expression was prognostic of clinical failure and death from prostate cancer in univariate models. Further correlating with these data, high B7-H3 expression was also found to be prognostic of disease progression following salvage radiation therapy for recurrent prostate cancer after surgery.[10] In addition to these studies on primary prostate cancer tissue, B7-H3 protein expression has also been explored in metastasis and in prostate cancer treated with androgen deprivation. Here B7-H3 expression was identified in prostate cancer bone metastasis with a trend towards increased expression upon androgen deprivation.[11] In this work, neoadjuvant androgen deprivation did not appear to alter expression in prostatectomy tissue.

Here we studied *B7-H3* expression at the transcript level in two large prostatectomy series. Further, we used genome wide expression data to examine *B7-H3* expression among different molecular subtypes of prostate cancer and to correlate its expression with immune regulatory pathways and with androgen receptor signaling.

MATERIALS AND METHODS

Patient cohorts

Prostatectomy tissue was derived from two patient cohorts. The first included prostatectomy samples with associated genomic information from 2,111 patients prospectively submitted for clinical Decipher testing.[12] A second cohort included prostatectomy tissue from 670 patients that had undergone radical prostatectomies at the Johns Hopkins Medical Institute (JHMI). In the patients from JHMI, two case-cohort designs were used to investigate clinical outcomes: (1) a case-cohort based on 260 men with intermediate or high risk localized prostate cancer undergoing prostatectomy at JHMI and then followed expectantly until clinical metastasis,[13] and (2) a case cohort natural history study of 211 patients who had biochemical recurrence after prostatectomy but did not receive therapy until the time of metastasis.

Prostatectomy sample selection and processing—Specimen selection, RNA extraction and microarray hybridization was done in a Clinical Laboratory Improvement Amendments-certified laboratory facility (GenomeDx Biosciences, San Diego, CA, USA) as previously described.[13,14] Briefly, total RNA was extracted and purified using the RNeasy FFPE kit (Qiagen, Valencia, CA, USA). RNA was amplified and labeled using the Ovation WTA FFPE system (NuGen, San Carlos, CA, USA) and hybridized to Human Exon 1.0 ST GeneChips (Affymetrix, Santa Clara, CA, USA).

Quality control was performed using Affymetrix Power Tools, and normalization was performed using the Single Channel Array Normalization algorithm.[15] Gene expression was summarized using the Affymetrix core transcript cluster and corrected for batch effects using an empirical Bayes framework.

Chromatin immunoprecipitation—Publically available datasets of AR chromatin immunoprecipitation (ChIP)-Seq experiments were analyzed using IGV.[16,17] A putative androgen-induced AR-binding site was identified upstream of the *B7-H3 (CD276)* gene. Chromatin immunoprecipitation experiments were performed as described previously.[18] In brief, formaldehyde cross-linked LNCaP cells were subjected to immunoprecipitation with AR specific antibodies (Millipore, Darmstadt, Germany) or control IgG (Cell Signaling Technologies, Danvers, MA, USA) after 8 h of 100 n_M dihydrotestosterone (DHT, Sigma Aldrich, St. Louis, MO, USA) or solvent control treatment. Enriched libraries were amplified using primers specific to the putative upstream regulatory site (upstream, F: 5′-GCTTTTATGAGCCTCCGTGA-3′; R: 5′-AGCACTGAGCCATTCACCTT-3′) and the transcriptional start site (TSS, F: 5′-CGTCCCTGAGTCCCAGAGT-3′; R: 5′-GGTTCCCGGGACTCCTGT-3′). Data are shown as relative enrichment normalized to input DNA. Primers specific to the *KLK3* locus (which encodes for PSA and harbors a well-characterized AR-binding site) were used as a control.

Androgen-dependent expression of *B7-H3* in prostate cancer cell lines—LNCaP cells were grown in RPMI and either charcoal stripped fetal bovine serum (FBS) (deprived of androgens), FBS (contains androgens) or charcoal stripped FBS supplemented with DHT. Cells were harvested and *B7-H3* expression was assayed using quantitative reverse transcription PCR with Taqman assays. All experiments were done in quadruplicate. LNCaP cells were obtained from ATCC, authenticity was validated by STR profiling and cells were confirmed as mycoplasma free by the MycoAlert Mycoplasma Detection Kit (Lonza, Basel, Switzerland).

Statistical analysis

B7-H3 expression distribution was compared with the expression of 3 other known checkpoint molecules. Survival analysis was performed in the JHMI cohorts to evaluate the prognostic value of *B7-H3* and to compare the prognostic value of the 4 ligands. Kaplan–Meier curves were constructed with appropriate weighting of the cohorts to account for the case-cohort study design.[19] To test the association of *B7-H3* expression with clinicopathologic variables pair-wise comparisons using Wilcoxon rank-sum test and, where appropriate, adjusted *P*-values via Bonferroni correction. Associations of *B7-H3* expression with molecular subtypes (ERG+, ERG-ETS+, ERG-SPINK1+ and TripleNeg)[20] were evaluated using Pearson's correlation. The association of *B7-H3* expression with AR was assessed using Pearson's correlation. Statistical analyses were performed in R, and all statistical tests were two-sided using a 5% significance level.

Functional characterization and GSEA

Associations of *B7-H3* expression with 104 genes which are a part of Nanostring's PanCancer Immune Profiling Panel were evaluated using Pearson's correlation and *P*-values were adjusted using Holm's method. Genes were ranked based on their Pearson's correlation to *B7-H3* in the prospective cohort and gene set enrichment analysis (GSEA) was carried out to identify gene sets associated with *B7-H3* by using the GSEA analysis tool downloaded from the Broad Institute website (http://www.broadinstitute.org/gsea/index.jsp). The curated gene sets of the Molecular Signature Database (MSigDB) version 4.0 was used for

enrichment. The false discovery rate for GSEA is the estimated probability that a gene set with a given NES (normalized enrichment score) represents a false-positive finding, and an false discovery rate<0.25 is considered to be statistically significant for GSEA. The Database for Annotation, Visualization and Integrated Discovery (DAVID) was used to evaluate molecular concepts enriched in genes which were highly correlated with *B7-H3* (ref. 21).

RESULTS

Relationship of *B7-H3* and Immune ligands to pathologic and clinical correlates

When compared with benign prostatic tissue, *B7-H3* expression is significantly higher in prostate cancers (P <0.04; Supplementary Figure 1).[22] In order to further examine *B7-H3* expression in prostate cancer, we analyzed 2111 prostatectomy samples for which microarray data were available. Median B7-H3 expression was within the top 19th percentile of genes expressed in prostate cancer and was 2.5-fold higher than the median expression for all genes. By contrast, and concordant with previous studies, *PD-L1*, *PD-L2* and *B7-H4* were not highly expressed in prostate cancer (Figure 1a). Expression of *B7-H3* varied significantly but modestly according to stage and grade, with median expression increasing among those men with primary Gleason pattern 4 or Gleason sum 8–10 disease or those with non-organ confined disease (P <0.001 for both; Figure 1b and Supplementary Table 1). *B7-H3* expression was significantly increased in castrate resistant metastatic prostate cancer when compared with localized disease (Figure 1c).

To determine the correlation between increased *B7-H3* expression and oncologic outcomes following surgery we performed Kaplan–Meier analysis for metastatic events based on median split of B7-H3 expression in cohorts for which long-term follow-up was available. Among men who underwent prostatectomy and then had no further treatment until the time of metastasis, higher *B7-H3* expression (but *not PD-L1*, *PD-L2* or *B7-H4* expression) was significantly associated with metastatic outcome (Figure 2a). We additionally analyzed a separate case-cohort study of men who underwent radical prostatectomy and all of which developed biochemical recurrence but had no further treatment until metastatic progression. Among these men, who are higher risk of metastatic progression, increased *B7-H3* expression also significantly correlated with the development of clinical metastasis (Figure 2b). To determine whether *B7-H3* expression is independently associated with outcome, we performed mutli-variable analysis including standard prognostic variables for each cohort. These studies showed that *B7-H3* is not an independent predictor of outcome, likely due to the positive correlation of *B7-H3* expression levels with grade and stage (Supplementary Table 2).

Relationship of *B7-H3* to androgen receptor signaling

We next investigated the relationship between *B7-H3* expression and molecular subtypes of prostate cancer.[20] *B7-H3* expression was relatively elevated among patients with ERG+ disease and decrease among triple negative patients (Figure 3a). A positive correlation between ERG+ status and *B7-H3* expression suggested a relationship between *B7-H3* and androgen receptor signaling. In regards to correlation of *B7-H3* expression with AR

expression itself, we also found a positive correlation (Figure 3b), with B7-H3 being among the most correlated genes with AR expression (top 15th percentile) and visa versa (top 21st percentile) (Figures 3c and d). A similar finding was demonstrated in the JHMI cohorts with Pearson correlation coefficients of 0.47 and AR falling in the top 6th percentile of genes correlated with B7-H3 (Supplementary Figure 2). In gene set enrichment analysis, the androgen receptor signaling pathway was positively correlated with *B7-H3* expression (normalized enrichment score = 1.62 and 1.90 $P = 0.05$ and <0.001 for the prospective and JHMI cohorts, respectively) (Figure 4). We next investigated whether AR would show androgen induced binding at the B7-H3 (*CD276*) locus. Mining publically available ChIP-Seq data sets, we identified an AR-binding site upstream of B7-H3 (ref. 16). To corroborate this finding we performed AR ChIP in LNCaP cells and evaluated AR binding to a putative B7-H3 upstream regulatory element and the B7-H3 promoter in the absence or presence of androgen. Androgen stimulation resulted in a robust induction of AR binding to the B7-H3 upstream region, with enrichments similar to other well-characterized AR-binding sites (*KLK3/PSA* enhancer) (Figure 5 and Supplementary Figure 3).

To address the functional consequence of AR binding upstream of *B7-H3* we evaluated the expression of *B7-H3* under different conditions in the LNCaP prostate cancer cell line. LNCaP cells grown in RPMI supplemented with FBS (which contains androgens) show low expression of *B7-H3* compared with cells grown in medium supplemented with charcoal stripped FBS (deprived of androgens). Conversely, expression of *B7-H3* was greatly reduced in LNCaP cells grown in charcoal stripped FBS, which was supplemented with DHT. These findings, shown in Figure 5c, suggest that androgen receptor binding negatively regulates *B7-H3* expression. This finding is further supported by *in silico* analyses of publically available datasets supporting the notion that *B7-H3* expression is suppressed by AR (Supplementary Figure 4).

Given the relationship between *B7-H3* and AR, we conducted an exploratory analysis using genes having a Pearson's correlation coefficient >0.5 between *B7-H3* and *AR* and used DAVID to functionally cluster those genes. Enriched clusters included genes involved in cell cycle, cell differentiation, proteolysis, apoptosis, splicing and DNA repair, as well as those associated with the androgen receptor and WNT signaling (Supplementary Figure 5).

Gene set enrichment analysis and immune gene correlations with *B7-H3* expression

In order to define putative relationships and functions for B7-H3, we performed unbiased gene set enrichment analysis using data derived from the prostatectomy tissue of the 2111 patients in the prospective cohort. Selected top positively enriched gene sets are shown in Table 1 with a complete list in Supplementary Table 3. No significantly negatively correlated gene set was found. Because of B7-H3's role in immune modulation we were particularly interested in the enrichment of immune pathways. Significantly enriched immune related gene sets included transforming growth factor (TGF)-β signaling and IL2-STAT5 signaling pathways (NES 1.6 and 1.6 respectively, adj P <0.05 for both). To further explore the associations between *B7-H3* and immune related genes we investigated the correlation between *B7-H3* gene expression and expression of genes within a previously described immune panel (http://www.nanostring.com/products/pancancer_immune/, Supplementary

Table 4). Supporting the enrichment of the TGF-β signaling pathway, we saw positive correlations between *B7-H3* expression and *SMAD2*, *IL17RA* and *RORC* (Figure 6).

DISCUSSION

Recent successes in the treatment of melanoma, bladder cancer, lung cancer and kidney cancer via immune checkpoint blockade has galvanized efforts to understand the interplay between tumors and the immune system and to develop novel therapeutic strategies in immuno-oncology.[23–25] Although immunotherapies have demonstrated some efficacy in prostate cancer, to date, their benefit has been somewhat marginal.[26,27] B7-H3, is an attractive target for further study in prostate cancer based on immunohistochemical data suggesting its increased expression in aggressive and castrate resistant metastatic prostate cancer and its homology to PD-L1 suggesting a potential role in immune modulation.[8–11] Indeed, recently reported phase I results with Enoblituzumab (MGA271), an Fc-optimized monoclonal antibody targeting B7-H3 shoed clinical activity in advanced malignancies including prostate cancer.[28] Here we examined *B7-H3* at the level of gene expression and performed exploratory analyses to generate hypotheses regarding its relationship to the androgen axis and immune regulatory pathways in prostate cancer.

We found that *B7-H3* was expressed in the majority of patients with localized prostate cancer. This was in contrast to *PD-L1, PD-L2* and *B7-H4*, all of which showed low levels of expression and parallels findings of B7-H3 and other B7 homologs studied at the protein level. Similar to what is found in immunohistochemical studies, *B7-H3* expression was associated with higher grade and stage disease, castrate resistant prostate cancer, and early disease progression after local treatment. Our data thus supports the increased expression of B7-H3 in aggressive disease and suggests that mRNA expression levels of *B7-H3* may potentially act as a good surrogate for protein level expression.

It is worth noting that a number of animal studies suggest that B7-H3 has a stimulatory role in driving anti-tumor immunity, such that, contrary to the findings presented here—increased B7-H3 expression might be expected to correlate with improved clinical outcome. Indeed, studies of forced expression in a lymphoma model,[6] a mastocytoma model[7] and a colorectal cancer model[29] suggest that B7-H3 interacts with a stimulatory receptor on CD8 T cells, and that this receptor may be TLT-2 (ref. 30). Data in certain human cancers are consistent with an anti-tumor role for B7-H3; in both gastric[31] and pancreatic cancer[32] expression is associated with improved survival. In other human cancers such as renal cell carcinoma[33] and prostate cancer (ref. 8 and data shown here), B7-H3 expression is associated with a poor outcome. The reasons for this apparent discrepancy are not immediately obvious, but could potentially be explained by differential expression of activating versus inhibitory B7-H3 receptors on immune cells, or by differential infiltration of tumor subtypes with sets of immune cells. For example, expression of a stimulatory B7-H3 receptor on pro-tumor M2 macrophages or myeloid derived suppressor cells could occur in tumors in which B7-H3 expression is associated with poor outcome—here the stimulation of suppressive cells could lead to tumor progression. Conversely, in other tumor types the B7-H3 receptor could be expressed predominantly on tumor-specific CD8 T cells, so that receptor engagement could lead to an anti-tumor effect, as has been observed in most animal studies. This dual effect is

not specific to B7-H3, infiltration of tumors with regulatory T cells is associated with poor outcome in most tumors,[34] but not in colorectal cancer, where it is associated with an improved outcome.[35] Identification of the human B7-H3 receptor(s) would provide crucial insights in terms of resolving these issues.

B7-H3 expression was strongly correlated with ERG positivity and negatively correlated with triple negative tumors (ERG negative, ETS negative and SPNIK negative). This suggested a putative relationship between *B7-H3* and androgen signaling, which was reinforced by gene set enrichment of androgen receptor signaling. In addition, a possible androgen response element was identified in the 5′ UTR of *B7-H3*, with occupancy being dependent on the presence of androgens. Taken together, these data suggest that *B7-H3* expression may be regulated by the androgen signaling axis and indeed, in LNCaP cells we observed a suppressive effect of androgens on *B7-H3* expression.

Whether *B7-H3* has a proinflammatory or immunosuppressive role in human prostate cancer remains unclear. An examination of *B7-H3* expression in relation to other immune pathways and genes suggested an association with TGF-β signaling. Further, a positive correlation with *RORC* and *IL17* may be suggestive of increased *B7-H3* expression being related to a Th17 type response (as seen in animal models) and possibly with an increase in regulatory T cells.[4] As B7-H3 directed therapy enters into clinical study in human prostate cancer it will be critical to perform correlative science to help elucidate which molecular interactions can be capitalized on, particularly for combination therapy trials.

In summary, we find that *B7-H3* expression is present in the majority of localized prostate cancer cases, with levels of *B7-H3* increasing with tumor extent and aggressiveness. *B7-H3* is potentially androgen regulated and related to the TGF-β signaling pathway, however these associations require further study. *B7-H3* expression characteristics may help guide patient selection for clinical trials, particularly to anti-B7-H3 antibodies such as enoblituzumab.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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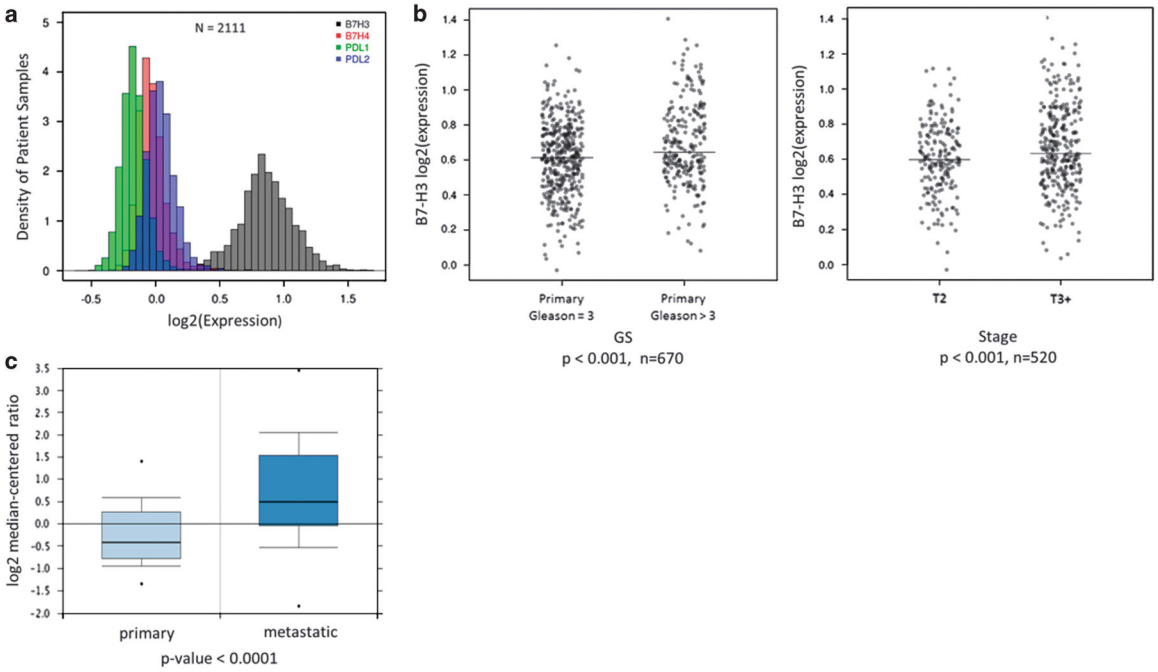

Figure 1.

(**a**) Expression distributions of *B7-H3*, *B7-H4*, *PD-L1* and *PD-L2* in a prospective radical prostatectomy (RP) cohort ($n = 2111$) (**b**) Dot plots showing *B7-H3* expression is higher in more aggressive tumors as it is significantly associated with pathologic Gleason grade and tumor stage. (**c**) Box plots showing *B7-H3* expression is associated with metastasis as expression is higher in metastatic tumors vs to primary tumors. Data for this comparison were derived from Oncomine consisting of 59 primary tumor samples and 34 metastatic castration resistant prostate cancer tumor samples ($P < 0.0001$). GS, Gleason score.

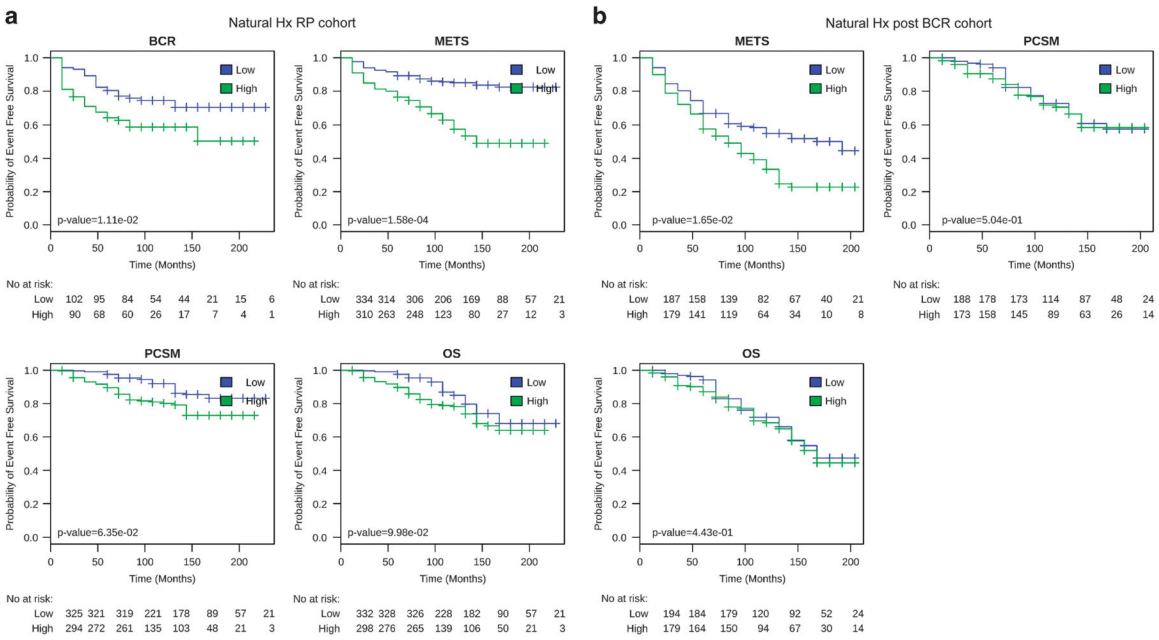

Figure 2.

(**a**) Kaplan–Meier curves for biochemical recurrence (BCR), clinical metastasis (METS), prostate cancer specific mortality (PCSM) and overall survival (OS) in a natural history prostate cancer radical prostatectomy (RP) cohort. (**b**) Kaplan–Meier curves in a natural history RP cohort of patients that developed BCR after RP.

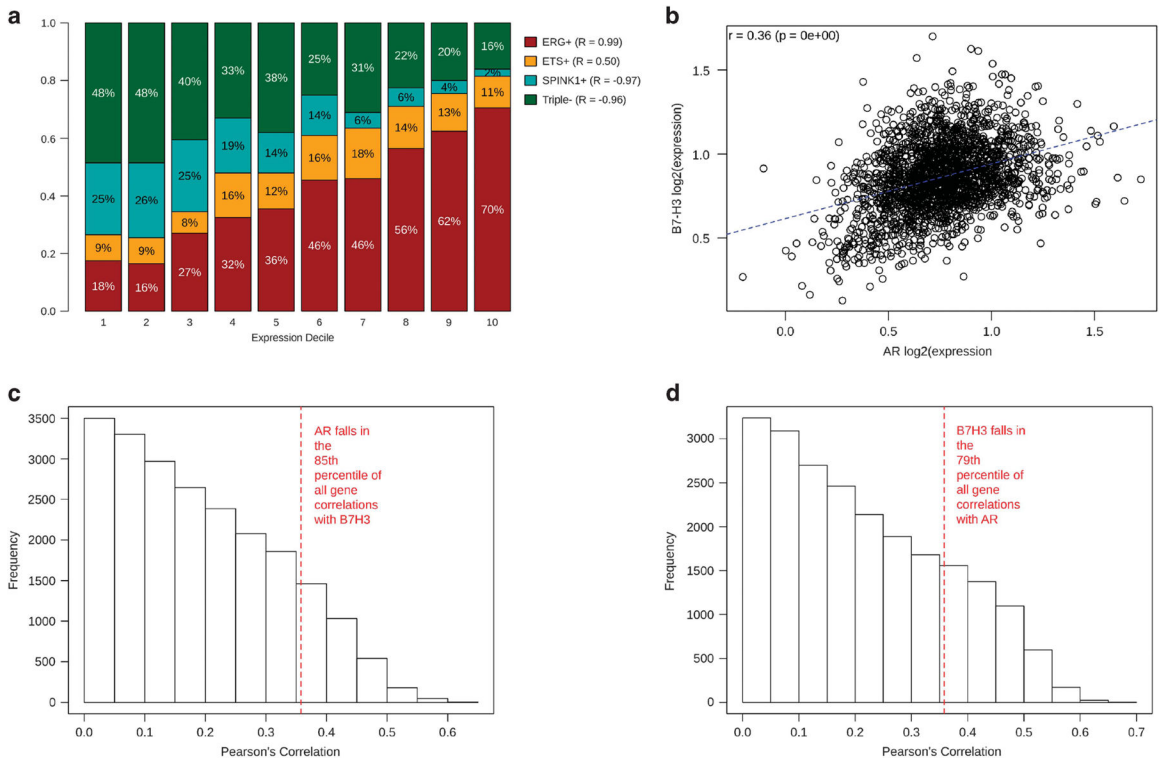

Figure 3.

(**a**) *B7-H3* expression trends with the molecular subtype in samples from a prospective RP cohort (*n* = 2111) (**b**) *B7-H3* expression positively correlates with *AR* expression when measured in a prospective cohort (*n* = 2111, R = 0.36) (**c**) *AR* is among the most correlated genes with *B7-H3* (85th percentile) (**d**) *B7-H3* is among the most correlated genes with androgen receptor (AR; 79th percentile). RP, radical prostatectomy.

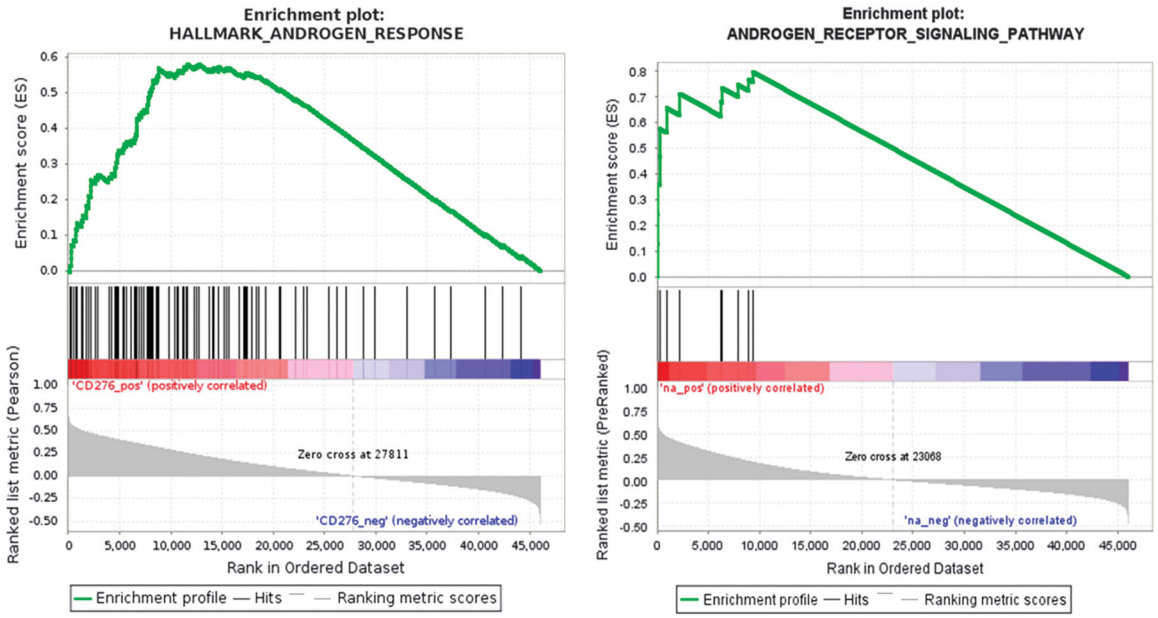

Figure 4.

Gene set enrichment analysis in the prospective cohort (left) and Johns Hopkins Medical Institute (JHMI) natural history cohorts (right) shows the androgen receptor signaling pathway is positively correlated with B7-H3 expression.

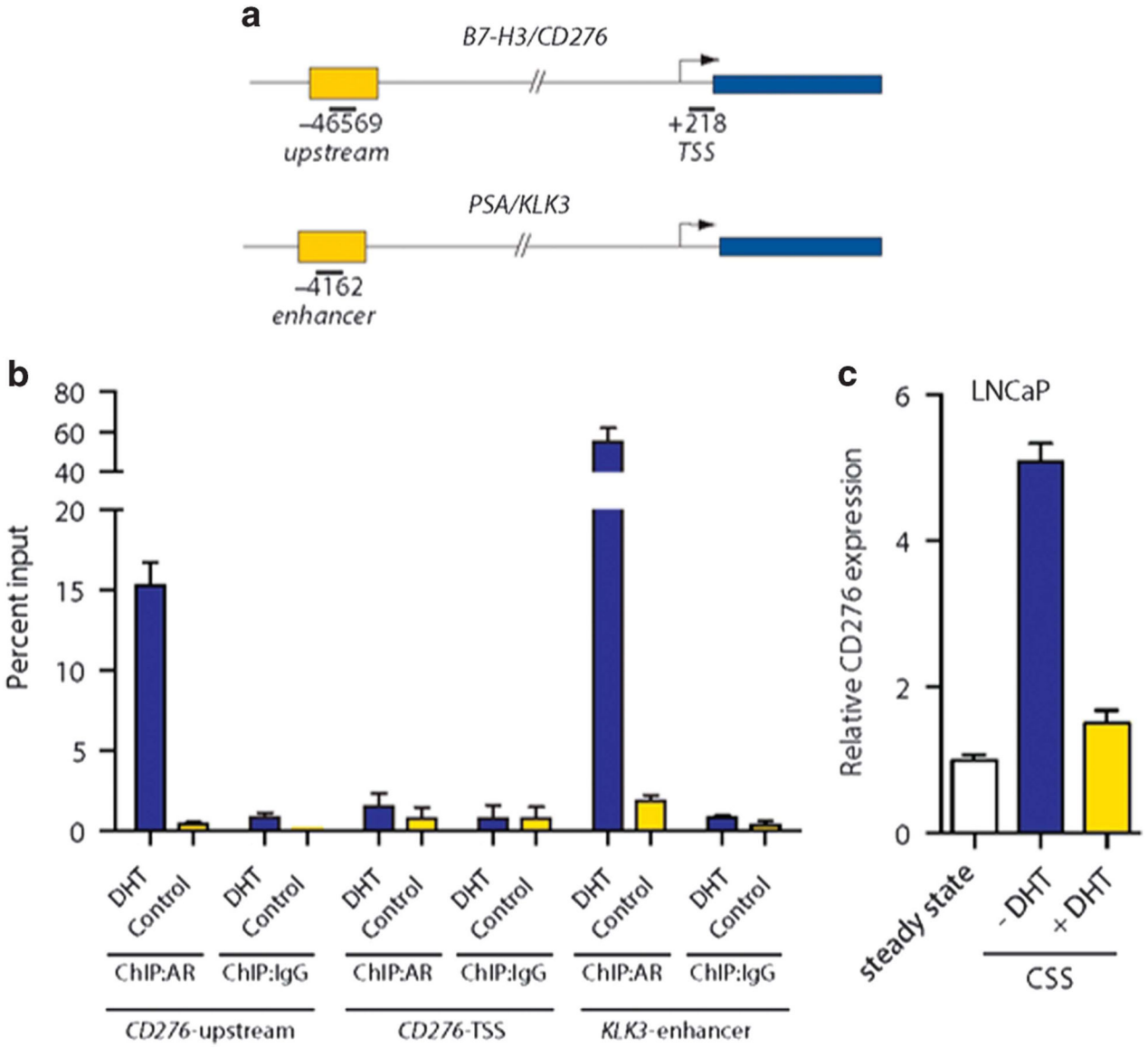

Figure 5.

Androgen-induced binding of the androgen receptor (AR) to a putative regulatory element upstream of *B7-H3/CD276*. (**a**) Schematic of primer locations (bars indicate primer positions relative to transcriptional start sites (TSS)). (**b**) AR binding sites were identified by mining previously published ChIP-Seq data. Chromatin precipitation was performed using LNCaP cells grown in the absence of androgens and treated for 8 h with 100 n_M DHT or solvent control. Cell lysates were incubated with anti-AR or IgG control antibodies and precipitated DNA was analyzed using primers specific to an upstream region of *B7-H3/CD276* or the *B7-H3/CD276* promoter (TSS). Enrichment at the *KLK3/PSA* enhancer is shown as a positive control. Data are shown normalized to total DNA input for each amplicon. (c) Expression of *B7-H3* as assayed by quantitative reverse transcription (qRT-PCR) in LNCaP cells grown in RPMI supplemented with FBS (which contains androgens),

RPMI supplemented with charcoal stripped FBS (deprived of androgens), or RPMI with charcoal stripped FBS, which was supplemented with DHT. FBS, fetal bovine serum.

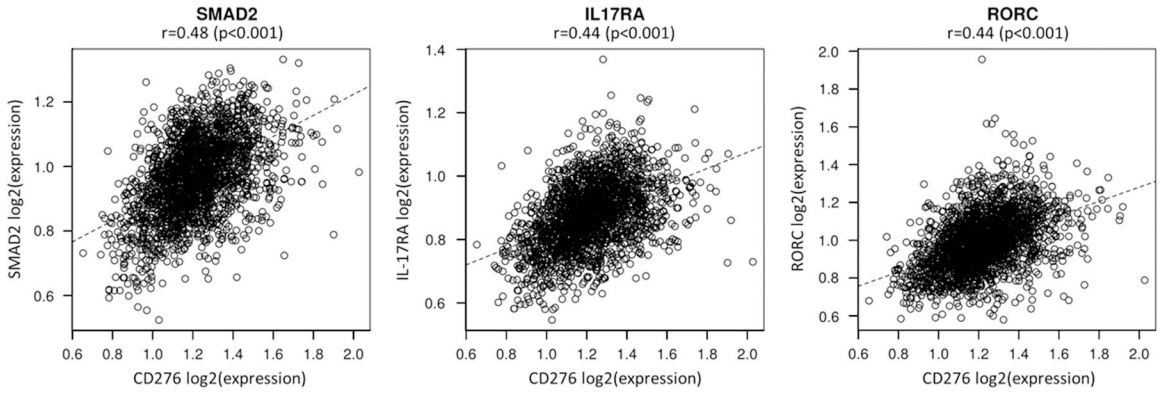

Figure 6.

Pearson's correlation analysis shows positive correlations between *B7-H3* expression and *SMAD2, IL17RA* and *RORC* in the prospective cohort.

Table 1.

Enriched gene sets

Gene set	Size	Enrichment Score	Normalized Enrichment Score	FDR q-val
PI3K_AKT_MTOR_SIGNALING	105	0.64	1.91	0.02
MTORC1_SIGNALING	199	0.63	1.83	0.01
MYC_TARGETS_V1	199	0.72	1.80	0.02
P53_PATHWAY	199	0.56	1.79	0.02
WNT_BETA_CATENIN_SIGNALING	42	0.62	1.76	0.02
NOTCH_SIGNALING	32	0.65	1.72	0.02
TGF_BETA_SIGNALING	54	0.61	1.64	0.03
IL2_STAT5_SIGNALING	199	0.49	1.60	0.04
HEDGEHOG_SIGNALING	36	0.52	1.59	0.05
ANDROGEN_RESPONSE	100	0.58	1.58	0.05

Abbreviation: FDR, false discovery rate